

Chromatin Insulator Elements Block Transgene Silencing in Engineered hESC Lines at a Defined Chromosome 13 Locus.

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Public Summary:

Lineage reporters of human embryonic stem cell (hESC) lines are useful for differentiation studies and drug screening.

Scientific Abstract:

Lineage reporters of human embryonic stem cell (hESC) lines are useful for differentiation studies and drug screening. Previously we created reporter lines driven by an elongation factor 1 alpha (EF1alpha promoter at a chromosome 13q32.3 locus in the hESC line WA09 and an abnormal hESC line BG01V in a site-specific manner. Expression of reporters in these lines was maintained in long-term culture at undifferentiated state. However, when these cells were differentiated into specific lineages, reduction in reporter expression was observed, indicating transgene silencing. To develop an efficient and reliable genetic engineering strategy in hESCs, we used chromatin insulator elements to flank single-copy transgenes and integrated the combined expression constructs via PhiC31/R4 integrase mediated recombination technology to the chromosome 13 locus precisely. Two copies of cHS4 double insulator sequences were placed adjacent to both 5' and 3' of the promoter reporter constructs. The green fluorescent protein (GFP) gene was driven by EF1alpha or CMV early enhancer/chicken beta actin (CAG) promoter. In the engineered hESC lines, for both insulated CAG-GFP and EF1alpha-GFP, constitutive expression at the chromosome 13 locus was maintained during prolonged culture and in directed differentiation assays towards diverse types of neurons, pancreatic endoderm and mesodermal progeny. In particular, described here is the first normal hESC fluorescent reporter line that robustly expresses GFP in both the undifferentiated state and throughout dopaminergic lineage differentiation. The dual strategy of utilizing insulator sequences and integration at the constitutive chromosome 13 locus ensures appropriate transgene expression. This is a valuable tool for lineage development study, gain- and loss-of-function experiments, and human disease modeling using hESCs.

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